Setting up a new experiment on the Quanteon

NOTE: Plates for the Quanteon loader have to be calibrated before use. If the instrument is not calibrated for your plate, please contact FACS Core.

The following plates have already been calibrated.

Type	Shape	Material	Company	Ref
96 well	U-bottom	polystyrene	FALCON	353910
96 well	U-bottom	polystyrene	NUNC, Thermo Scientific	163320
96 well	V-bottom	polystyrene	NUNC, Thermo Scientific	249935
96 well	V-bottom	polystyrene	NUNC, Thermo Scientific	249570
96 well	Flat-bottom	polystyrene	NUNC, Thermo Scientific	439454
96 well	V-bottom	polystyrene	Sarsted	82.1583
96 well	U-bottom	polystyrene	Sarsted	82.1582
96 well	U-bottom	Polypropylene	NUNC	
24 well	Flat-bottom	polystyrene	NUNC, Thermo Scientific	142475
48 well	Flat-bottom	polystyrene	NUNC, Thermo Scientific	150687

- **Tubes.** 5 ml tubes differ between different companies.
  We know that the following brand works well:
  Falcon: ref. 352054
  Beckman Coulter: ref. 2523749 (polypropylene tubes, good if you have sticky cells and you want to avoid activating your cells)
  Falcon: ref 352235 (Tubes including a 0.45µm filter – recommended for tumor samples)
  Do NOT use 5 ml tubes from Sarsted.

1) Log in to your account
2) **NovoExpress automatically opens a new experiment "untitled". Save the experiment in your own experiment folder** (File/Save)
   NB!: Settings will be the same as for the recently run experiment!
3) Choose in Plate Manager: Tubes or plate
4) Create a specimen containing a sample in A1, by clicking in A1 and clicking the "+ tube" in the Plate Manager window.

5) Select mixing interval, duration, speed and acceleration if you do not want the default settings and the rinse conditions in the Plate Manager window.

   If you use DNA stains, rinse 3 cycles between samples to minimize carry over.

6) Uncheck Abs Count, if you do not need it (20µl extra sample will be taken up, but not analysed when "Abs count" is checked on)

7) Decide if you want to recover remaining sample.

8) Go to Parameters window and select your parameters for this experiment

9) Be aware that many parameters can be measured both of the blue and the yellow laser. Be sure to choose the correct parameter

10) Create two dot plots: FSC-H x SSC-H and FSC-A x SSC-A

11) Select speed and stop conditions and set a low threshold based on the guidelines (on the wall above the instrument).

12) Place a (negative control) cell suspension in position A1.

13) Run Single Well/Tube: A1

14) After running:
    Adjust FSC threshold value just beneath your cells – not too close. You can click on the text "Adjust on plot" to visualize the threshold in a FSC-H x SSC-H plot (the area/events below the threshold will become gray). Clicking where you want the threshold will give you the corresponding threshold value.

    This value is not automatically transferred to other samples.

    Note: Threshold is based on height; therefore, use the FSC-H x SSC-H dot plot to set your threshold.

15) Select the position of your samples in the Plate Manager window and click "+Tube"

16) Go to the Work List.
    Name your Specimen and Samples (You can also name your samples while they are running).

17) Double-click relevant fields in the worklist to modify Stop Condition, Threshold and Flow Rate for one sample. Or copy paste from one sample to the rest of your samples. Check that the correct parameters are turned on/off for all samples.

18) Save (Apply Modification) and close your Worklist

19) In Experiment Manager: You can copy all of your plots from one sample to other samples by dragging the analysis from one sample to the experiment, the specimen or tube of interest.

20) If you are performing **Auto Compensation** press that button.

A pop-up window appears. Select your parameters. Be aware that the parameter names should be exactly the same as in your samples. Click OK and a Compensation Specimen appears.
It is possible to rename it in the worklist and change the threshold for only this specimen, if you are doing compensation with beads.

After running compensation beads or single stained cells, check all gates, drag the blue "Compensation" in the compensation specimen to your experiment, specimen or single tube of interest.

21) Create the dot plots and gates needed for your analysis (you can also do this while your samples are running)

22) Run your samples: Click "Run Plate" in the Cytometer Control window and select in the popup window which tubes or wells to run

23) While running you can set up your gating strategy and/or name your samples.

24) When you look at fluorescence parameters in NovoExpress – always use **biexponential scaling**. Right-click on the axes and change to biexponential

25) After running samples:

Export your data as FCS files: Right click the experiment or specimen/Export/Export to FCS files and then choose OK

If you want to export statistic tables use export CSV files

Transfer your data to your au-server: see "Connect and save data to Network Share" and delete your FCS files from the computer.

Additional notes:

- If you want to re-use a sample-setup from one experiment to another, you can open a new experiment from an old one: File/New/New from Experiment File.
- You can save your compensation, analysis and parameters together as a template: Right click on a tube and export as template. This template can be applied to a sample, a specimen or an experiment in the future.
- The word "template" does not mean the same in the NovoExpress as in Diva software